Hematologic and Biochemical Reference Intervals for Captive Asian Elephants *(Elephas maximus)* in Thailand

Thittaya JANYAMETHAKUL [1]   Supaphen SRIPIBOON [1]   Chalermchat SOMGIRD [1,2]
Pornsawan PONGSOPAWIJIT [1,2]   Veerasak PANYAPORNWITHAYA [3]
Sarisa KLINHOM [1]   Jarunee LOYTHONG [4]   Chatchote THITARAM [1,2] ✎

[1] Center of Excellence in Elephant Research and Education, Chiang Mai University, Chiang Mai, 50100, THAILAND
[2] Department of Companion Animals and Wildlife Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, THAILAND
[3] Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, THAILAND
[4] Heamatology Laboratory of Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, THAILAND

Abstract

Species specific blood value reference intervals are needed for the proper diagnosis, and treatment of disease, appropriate for specific populations, because age, sex, management, exercise and geographical location can all affect hematological values. The aim of this study was to establish a set of hematology and blood chemistry reference intervals for captive Asian elephants. Blood samples were collected from 149 healthy Asian elephants in 15 tourist camps in Northern Thailand. Hematological and biochemical parameters were determined. The results showed similarity of haematological and blood chemistry range to others previously published. There were no sex differences for most hematological parameters except some parameters were different i.e. MCV, MCHC, BUN, AST, and ALP. The hematology and blood chemistry reference intervals of our study can be used as the reference for hematological analysis in Thailand, and several Asian elephant range countries and zoos.

Keywords: Asian elephant, Blood parameter, Blood chemistry, Hematology, Reference intervals

Tayland'da Evcil Asya Filinin *(Elephas maximus)* Kan ve Biyokimyasal Referans Aralıkları

Özet

Türlerin spesifik kan değerleri referans aralıklarının bilinmesi uygun teşhis ve hastalıkların tedavisi için gerekli olup yaş, cinsiyet, bakım, egzersiz ve coğrafi bölge kan değerlerini etkilemektir. Bu çalışmanın amacı evcil Asya filinin hematoloji ve kan kimyası değerlerini ortaya koymaktır. Kuzey Tayland'daki 15 turist kampında yer alan 149 adet sağlıklı Asya filinden kan örnekleri toplandı. Kan ve biyokimyasal parametreler belirlendi. Sonuçlar daha önceden bildirilmiş olan kan ve biyokimyasal değerler ile benzerlik gösterdi. Çoğu kan parametreleri için cinsiyetin bir farkı gözlenmezken MCV, MCHC, BUN, AST ve ALP gibi bazı parametrelerde fark gözlemlendi. Mevcut çalışmada elde edilen hematoloji ve kan kimyası referans aralıkları Tayland'da ayrıca Asya filinin bulunduğu ülke ve hayvanat bahçelerinde kan analizleri için referans olarak kullanılabilir.

Anahtar sözcükler: Asya fili, Kan parametreleri, Kan kimyası, Hematoloji, Referans aralıkları

INTRODUCTION

Accurate hematologic and biochemical reference intervals are useful for evaluating the health status of animals and for proper diagnosis of disease and evaluation of treatment efficacy. The Asian elephant *(Elephas maximus)* is an endangered species, and in Thailand is an important part of the country's history and culture. Today, they are also important economically, as most captive elephants in Thailand are used primarily for tourism. The need for proper veterinary care of tourist camp elephants is increasing, and more veterinarians are relying on evaluations of blood

✎ İletişim (Correspondence)

☎ +66 53 948097, Fax: +66 53 948065

✉ chatchote.thitaram@cmu.ac.th

tests to assist in diagnosis and treatment of disease.

Although hematology and blood chemistry data exist for both African and Asian elephants, overall the ranges tend to be very broad [1-4], which makes interpretation difficult. Furthermore, values may not be relevant across all populations because factors such as age, sex, management, exercise, as well as geographical location can affect values (e.g., horse [5], human [6]). Yaqub [7] reported that in farm animals, in the same species which locate in different farm could be found the unique of baseline or haematologic and blood chemistry. Therefore, the elephant in different country or feeding management like captive elephant in zoos and private camps may be different in hematologic and biochemistry value. Most blood parameter data in Asian elephants are based on samples collected from zoo elephants in North America and Europe [8], where the geographic, climate and management conditions are different from Thailand. There are some data on Asian elephants in range countries; e.g., India [2] and Sri Lanka [9,10], but there still are considerable differences in geography, where differs in the nutrition, which can lead to different blood parameters [11]. Physical exercise due to work and management, can cause stress between those elephants, which may affect the blood profiles [9,12]. Moreover, De Mel [9] found hematological ranges differed across several populations of Asian elephants in Sri Lanka. The aim of this study was to begin establishing a set of hematology and blood chemistry reference intervals for Asian elephant used in tourist camps in Thailand, and other Asian elephant range countries.

MATERIAL and METHODS

Animals

One hundred forty nine Asian elephants (41 males, 108 females) aged 3–60 years, from 15 tourist elephant camps in Chiang Mai, Thailand (latitude, 18°47'N; longitude, 98°59'E). These elephants were originally from various parts of Thailand. The age could not be identified in these elephants due to unclear birth date history. All elephants in this study were classified as healthy based on a physical examination by veterinarians experienced with elephants. All elephants performed work daily, either trekking or giving tourist rides or in an elephant show (not more than 6 hours per day). Most elephants were chained at night. For nutrition management, camps provided similar types and amounts of roughage, fruit, sugar cane and some vegetables. Bulls were provided a lower energy diet such as less roughage, winter melon or banana trunk, during the musth period which is a circannual period of anatomical, physiological and behavioural changes in mature Asian and African elephant bulls. During musth period, the bull shows temporal gland secretions (TGS), continuous urine dribbling (UD), increased aggression, and elevated serum androgen concentrations.

Sample Collection, Hematology and Blood Chemistry Analysis

Blood samples were collected from the auricular vein of these elephants during annual health checks. Blood samples were collected from the auricular vein of these elephants during annual health checks, mostly in the morning before activities. Blood was divided into 1) EDTA tube and gently mixed, and to 2) non-coagulation tube and was allowed to clot for ~1-2 h at room temperature before the serum was separated by centrifugation (1500 g) for 5 min. All samples were submitted to the Veterinary Diagnostic Laboratory of Faculty of Veterinary Medicine, Chiang Mai University, Thailand within 24 h of collection. Blood samples were analyzed by the Auto Hematology Analyzer (Mindray BC5300, Mindray Medical, Thailand) and by the Biochemical Analyzer Vitalab Flexor XL (Vital Scientific NV, Netherlands) except for white blood corpuscle differential count, which was manually assessed by a hematologist in the Veterinary Diagnostic Laboratory of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand. Hematology parameters i.e. packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin, Red Blood Cell count (RBC count), White Blood Cell count (WBC count), differential blood count, platelet count, and biochemical parameters i.e. Blood Urea Nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were analyzed.

Statistical Analysis

R program version 3.1.1 [13] was used for statistical analysis. Reference interval with 90% confidence intervals for each parameter was calculated using "referenceIntervals" package [14]. The option for outlier detection method was cook's distance. The student T-test was used to determine differences of blood parameters between males and females. The level of statistical significance was set at α<0.05.

RESULT

Reference range data for hematology and biochemistry evaluations of domestic Asian elephants in current study are shown in *Table 1*. Hematological parameters revealed no sex-associated clinically significant differences, except for MCV (*P*=0.011) and MCHC (*P*=0.002), which were higher in females than males, respectively. By contrast, BUN (*P*=0.001), AST (*P*=0.001) and ALP (*P*=0.049) were higher in males than females, respectively. White blood cell counts indicated that segmented neutrophils (47.5%) and lymphocytes (43.1%) predominated in this elephant population. The proportion of monocytes (monocyte and lobular monocyte) and eosinophils were found to be 7.4% and 2.05%, respectively, while observations of band neutrophils and basophils were rare.

Table 1. Reference Intervals of hematology and blood chemistry values for domestic Asian elephants in northern Thailand for both sexes

Parameter	Unit	Range (male, n= 41)	Range (female, n=108)	P-value
PCV	%	29.4-40.7	27.8-43	0.572
Hemoglobin	g/dL	9.8-15.2	10.1-15.6	0.162
RBC count	x 10^6 cell/μL	1.9-3.2	1.9-3.1	0.188
MCV*	fl	104-123.8	105.7-127.2	0.009
MCHC*	g/dL	29.9-38.9	32.1-38.7	0.015
WBC	cell/μL	7924.3-21890.3	7202.5-23220.5	0.657
Banded Neutrophil	cell/μL	Not found	Not found	-
Segmented Neutrophil	cell/μL	967.3-13425.8	828.7-13514.3	0.966
Lymphocyte	cell/μL	1672.4-11179.5	1064.1-12032.8	0.793
Monocyte	cell/μL	0-2391.4	0-3298	0.609
Bilobed monocytes	cell/μL	Not found	Not found	-
Eosinophil	cell/μL	0-866.8	0-1170	0.057
Basophil	cell/μL	0-142.6	0-36.3	0.067
Platelet	x 10^3 cell/μL	101.6-577.7	105.3-598.7	0.583
Reticulocyte	%	Not found	Not found	-
BUN*	mg/dL	3.1-27.2	4.2-19.7	0.004
Creatinine	mg/dL	0.7-2.2	0.9-1.8	0.358
AST*	U/L	4.8-56.3	10.1-39.6	0.013
ALT	U/L	0-4.9	0-5.6	0.826
ALP*	U/L	0-281.5	0-225.4	0.033
TP	g/dL	6.5-8.9	6.6-9.3	0.148

Asterisk showed significant difference of blood parameters between sexes ($P<0.05$)

DISCUSSION

In this study, most of the hematology and blood chemistry values were within the range of other reports for Asian elephants. There were some parameters which higher than other like the WBC count. This could be due to the inclusion of samples from different locations and management conditions, or from both healthy and unhealthy elephants.

Analysis of WBC found that segmented neutrophils and lymphocyte predominated as in previous reports [2,3,9]. However, we found that monocytes were in higher proportion than eosinophils, which were the same as Dastjerdi [15] reported that monocytes were the main WBC in juvenile elephants. This was contrasted with de Mel [9] and Salakij [3] who reported finding more eosinophils than monocytes. The variation of blood parameters of each study might be influent by various of laboratory error such as pre-analytical error; for instance, blood collecting method, blood sample preserving procedure before handing to laboratory, storage period until examination, and sample transportation. Process before handling to lab and storage period until examination also important due to damaged erythrocytes may swell during storage and transport, and this can increase the MCV which measured by automatic counters [16]. We assessed WBC counts manually because elephant cells, particularly monocytes that are bi-lobed, differ from those of humans, and using an automated human hematology analyzer can lead to unreliable results [17]. Also automated cell counting may report decreased values because of platelet or WBC aggregation, or fragile WBC.

Gender has been reported to be one of the factors that can affect hematological values in numerous species [18], although Silva and Kuruwita [19] and Salakij [3] also found no sex-associated clinically significant hematology differences in Asian elephants. In our study, MCV and MCHC were significantly higher in females than in males. In our study, the reproductive status of study elephants was unknown at the time of sampling, but camp records indicate most were not pregnant. Salakij [3] found leukocyte count and fibrinogen were significantly higher in males than females, although this difference was not observed in the present study. De Alwis [10] reported no significant difference of biochemical parameters between sexes; however, we found that male elephants had higher BUN levels than females. This may be due to a higher protein intake in bulls, which generally receive more food than females. The higher AST levels in bulls may be due to muscle activity; male elephants are stronger and work harder than females, especially in activities such as trekking (more tourist can be accommodated in the saddle), or kicking footballs

or logging demonstrations as part of the daily shows (personal communication). For ALP, which also was higher in males, Niemuller [20] suggested that musth bulls have higher concentrations of this enzyme than non-musth elephants. So in our study, the significant sex-different in ALP may indicate a musth condition for some bulls.

Gromadzka-Ostrowska [2] found hematocrit values, WBC, and neutrophil numbers of elephants in India were slightly higher, while RBC were lower during the winter due to poorer food quality. However, blood samples had been collected, and food with supplement were provide to these tourist elephants throughout the year; therefore, the influence of seasonal variation to hematological value was not high in these elephants. An animal's activities or type of work can affect health.

In this study, most of the referral ranges for hematology and blood chemistry values were similar to previous reports [17,19] which suggests they should be more appropriate for assessing health status in domestic Asian elephants in Thailand. There were gender difference for some of the blood parameters evaluated; therefore, sex of the animal should be taken into consideration for proper interpretation of blood data. Because of results of previous studies in other mammals, we suggest that further research in elephants should evaluate the effect of age and season on health parameters.

REFERENCES

1. White PT, Brown IR: Elephant blood haematology and chemistry. *Comp Biochem Phys B*, 65, 1-12, 1980. DOI: 10.1016/0305-0491(80)90107-8

2. Gromadzka-Ostrowska J, Jakubów K, Zalewska B, Krzywicki Z: Haematological and blood biochemical studies in female domesticated Indian elephants *(Elephas maximus* L.). *Comp Biochem Phys A*, 89, 313-315, 1988. DOI: 10.1016/0300-9629(88)91031-6

3. Salakij J, Salakij C, Narkkong NA, Apibal S, Suthunmapinuntra P, Rattanakukuprakarn J, Nunklang G, Yindee M: Hematology, cytochemistry and ultrastructure of blood cells from Asian elephant *(Elephas maximus)*. *Kasetsart J (Nat Sci)*, 39, 482-493, 2005.

4. Pintavongs W, Chueplaivej P, Boonyasart B, Kidyhoo S, Pravai W, Rattanakunuprakarn J, Ounsiri S, Lorsanyaluck B, Sunyathitiseree P, Jittapalapong S, Wajjwalku W, Thongtip N: Domestic elephant population structure and health status in Thailand. *J Kasetsart Vet*, 24 (1): 16-24, 2014.

5. Padalino B, Rubino G, Lacinio R, and Petazzi F: Observations on the hematology of standardbred horses in training and racing in southern Italy. *J Equine Vet Sci*, 34, 398-402, 2014. DOI: 10.1016/j.jevs.2013.07.018

6. Calkosinski I, Gamian A, Kobierska-Brzoza J, Fita K, Czajczynska-Waszkiewicz A, Majda J, Calkosinska M, Parulska O, Dobrzynski M: The influence of variable environmental factors on human's organism adaptive ability. *Adv Clin Exp Med*, 5, 519-527, 2009.

7. Yaqub Y: Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals: A review. *J Cell Anim Biol*, 7, 37-43, 2013. DOI: 10.5897/JCAB12.063

8. International Species Information System: Physiological reference values for elephant. http://www.elephantcare.org/isismain.htm, *Accessed*: 25.02.2016.

9. De Mel RK, Weerakoon DK, Ratnasooriya WD, and Dangolla A: A comparative haematological analysis of Asian elephants *(Elephas maximus* Linnaeus, 1758 [Mammalia: Proboscidea: Elephantidae]) managed under different captive conditions in Sri Lanka. *J Threat Taxa*, 6, 6148-6150, 2014. DOI: 10.11609/JoTT.o3761.6148-50

10. De Alwis GKH, Wijesekera RD, Vithana D, Neththasinghe N, and Ratnasooriya WD: Serum levels of some biochemical constituents of captive Sri Lankan elephants *(Elephas maximus maximus)*. *J Sci Univ Kelaniya*, 2, 105-114, 2005. DOI: 10.4038/josuk.v2i0.2749

11. Marchal J, Dorieux O, Haro L, Aujard F, and Perret M: Characterization of blood biochemical markers during aging in the Grey Mouse Lemur *(Microcebus murinus)*: Impact of gender and season. *BMC Vet Res*, 8, 211, 2012. DOI: 10.1186/1746-6148-8-211

12. Sutherland MA, Niekamp SR, Rodriguez-Zas SL, Salak-Johnson JL: Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds. *J Anim Sci*, 84, 588-596, 2006. DOI: 10.2527/2006.843588x

13. R Core Team: R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org, *Accessed*: 12.06. 2016.

14. Finnegan D: Package reference Intervals. http://cran.rproject.org/web/packages/referenceIntervals/index.html, *Accessed*: 12.06. 2016.

15. Dastjerdi A, Seilern-Moy A, Darpel K, Steinbach F, and Molenaar F: Surviving and fatal Elephant Endotheliotropic Herpesvirus-1A infections in juvenile Asian elephants - Lessons learned and recommendations on anti-herpesviral therapy. *BMC Vet Res*, 12, 178, 2016. DOI: 10.1186/s12917-016-0806-5

16. Salakij C: Veterinary Hematology. Department of Pathology, Faculty of Veterinary Medicine Kasetsart University, Bangkok, 2005. ISBN: 9745377406

17. Fowler ME, Mikota SK: Biology, Medicine, and Surgery of Elephants. Blackwell, United Kingdom, 2006. ISBN-13: 978-0-8138-0676-1; ISBN-10: 0-8138-0676- 3

18. Etim NN, Williams ME, Uduak A, Offiong EEA: Haematological parameters and factors affecting their values. *Agr Sci*, 2, 37-47, 2014. DOI: 10.12735/as.v2i1p37

19. Silva ID, Kurawita VY: Hematology, plasma, and serum biochemistry vales in domesticated elephants *(Elephas maximus ceylonicus)* in Sri Lanka. *J Zoo Wildlife Med*, 24, 434-439, 1993.

20. Niemuller C, Gentry PA, and Liptrap RM: Longitudinal study of haematological and biochemical constituents in blood of the Asian elephant *(Elephas maximus)*. *Comp Biochem Physiol A*, 96, 131-134, 1990. DOI: 10.1016/0300-9629(90)90053-U